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(71) Applicants: QUADRA LOGIC TECHNOLOGIES, INC. [CA/CA]; 520 West 6th Avenue, Vancouver, British Columbia V5Z 4H5 (CA). AMERICAN CYANIMID COMPANY [US/US]; 1 Cyanimid Plaza, Wayne, NJ 07470 (US).

(72) Inventors: BOWER, Robert, D. ; 5380 Williams Road, Richmond, V7E 1K1 (CA). STONEFIELD, Michael, D., L. ; 525 West 14th Avenue, No. 101, Vancouver, V5Z 1N9 (CA). WATERFIELD, Elizabeth, M. ; 4610 Blenheim Street, Vancouver, V6L 3A4 (CA). SAKAGUCHI, Edwin, M. ; 81 Walnut Hill Road, Bethel, CT 06801 (US).

(74) Agent: KODA, Steven, P.; Seattle Tower Building, 1218 Third Avenue, Seattle, WA 98101 (US).

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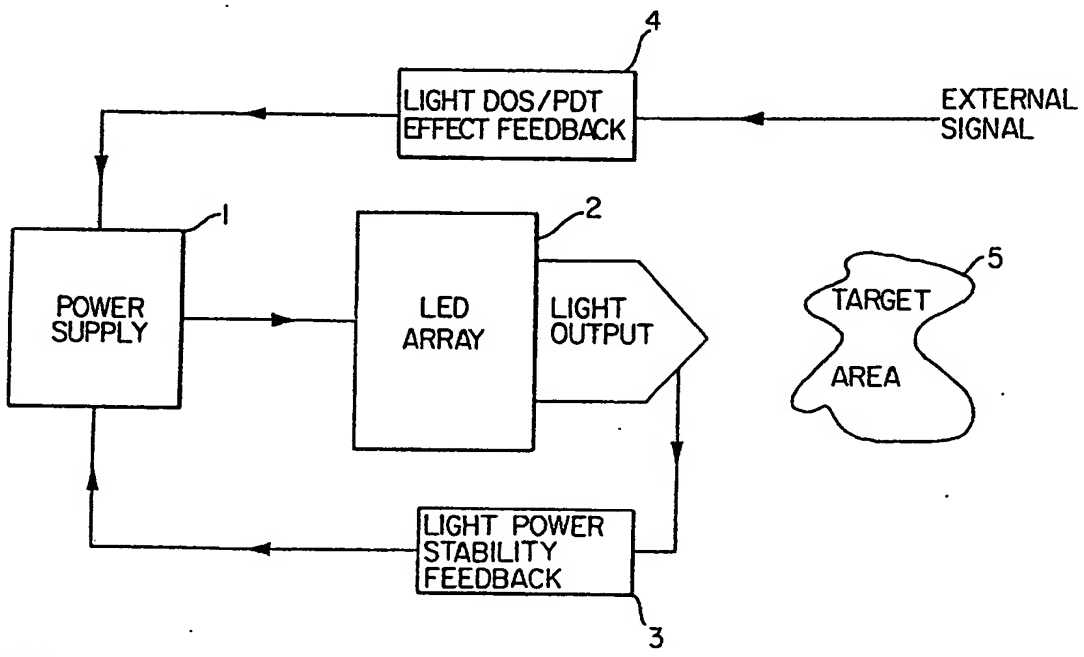

(57) Abstract

A method and system for activating photosensitizers for PDT *in vivo*, extracorporeally, and *in vitro*, where the light sources used are high-power light-emitting diodes (LEDs) and the LED wavelength band output is selected to access a given absorption band of the photosensitizer. The system includes a power supply (1), an array of LEDs (2), feedback loop (3) for monitoring LED output power and feedback loop (4) for monitoring light delivered to target area (5).

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HIGH-POWER LIGHT-EMITTING DIODES FOR PHOTODYNAMIC THERAPY

Field of the Invention

The invention generally relates to the use of high-power light-emitting diodes (LEDs) for use in photodynamic therapy (PDT). More specifically, high-power LEDs emitting a suitable wavelength band are used to activate photosensitive drugs for *in vitro*, extracorporeal, or *in vivo* PDT. The aim is to selectively impair or destroy targeted cancerous or otherwise undesirable tissues or cells while leaving healthy tissues or cells unaffected.

Background of the Invention

PDT or photodynamic therapy has become a recognized means of treating certain types of cancer. A review of many of the areas in which it has been applied is given by S.L. Marcus in 'Proceedings of the IEEE' (in publication). In essence, a photosensitizer such as PHOTOFRIN® porfimer sodium or BPD (benzoporphyrin derivative) is administered systemically to a patient with cancer. The drug distributes through the body in such a manner that it is found in higher concentrations at the diseased site. This can take several hours to a few days depending on the drug. A suitable light source is then used to activate the drug in the tissue.

An alternative scenario to the above would be one in which the drug is administered topically to the target tissue, e.g., a psoriatic lesion, site of viral infection, wart, or port wine stain. Once again, after

the drug has been taken up by the target site, the light source can be directed at the target to activate the drug, either via a fiber or by direct illumination.

Several photosensitizing compounds have been tested *in vivo* as potential clinical photosensitizing drugs, including PHOTOFRIN* and its precursor hematoporphyrin derivative, BPD, chloroaluminum phthalocyanine tetrasulfonate, zinc phthalocyanine tetrasulfonate, protoporphyrin IX, purpurin, merocyanine 540, methylene blue, tetraphenylporphyrin sulfonate, pheophorbide, monoaspartyl chlorin e6. These photosensitizers are activatable by light in the 500 nm to 780 nm range.

The mechanisms by which PDT works are complex, and the activation mechanisms may differ from one photosensitizer to another. However, a feature common to all of these photosensitizers is that they are activated by the absorption of light. For absorption to take place, the wavelength of light must coincide with a suitable photosensitizer absorption band. The absorption results in energy being deposited into the photosensitizer and subsequently initiates a series of chemical reactions which result in the death of cells. Since the energy is deposited only where the photosensitizer is located, only cells local to the photosensitizer are killed. The result of the cells' death depends on the treatment being performed. For example, in the case of photosensitizers such as PHOTOFRIN*, where the PDT is being done to eradicate a tumor, this cell killing appears to give rise to both localized destruction of the tumor tissues and to local vascular damage to the blood vessels supplying the tumor. The net result for correctly applied light and photosensitizer doses is for the tumor to be killed but not the surrounding healthy tissues.

To maximize the cell killing, the photosensitizer should have a strong absorption band, and the energy deposited into the photosensitizer should be efficiently converted into chemical reactivity. One mechanism by which this is achieved is outlined here. A photosensitizer in a singlet ground state is promoted into an excited singlet electronic state by photon absorption. This absorption will tend to be strong since it is a fully allowed transition. The excited singlet state will have a radiative lifetime on the order of a few nanoseconds. Vibrational relaxation within the excited singlet state to the lowest vibrational levels is rapid (10^{12} s^{-1}). The excited singlet state can emit a photon, undergo a spin-allowed internal conversion to the ground singlet state, undergo a spin-forbidden intersystem crossing into a triplet state, react, or transfer its energy to another molecule. By considering the electronic state's geometry and energy separation, the reactivity of the state, the effectiveness of potential quenchers and the spin orbit coupling between the singlet and triplet states, a photosensitizer is chosen such that significant production of the triplet state occurs. The triplet state will then vibrationally relax to the minimum energy of the triplet state. If the triplet state is of lower energy than the excited singlet state, the triplet state will not execute an intersystem cross-back into the excited singlet state. If there is large energy gap between the ground singlet state and the triplet state, then intersystem crossing from the triplet state into the ground state is typically slow. Since a triplet-to-singlet radiative transition is spin-forbidden, phosphorescence will not be a major loss mechanism. Therefore, the light energy is stored in the photosensitizer's metastable triplet state.

There are three major deactivation mechanisms for this triplet state: internal conversion into the ground singlet state, reaction of the triplet state, and energy transfer from the triplet state. If internal conversion into the ground state occurs, then the metastable triplet's energy is converted into energy in the singlet ground state. If vibrational relaxation in the ground state is fast, then the result is localized heating. This heating may kill cells by thermal effects. If the singlet state's energy is greater than bond-dissociation energy, then dissociation of the photosensitizer may occur resulting in the formation of radicals or radical ions, both of which may initiate chemical reactions resulting in the localized killing of cells. The second possibility is that the excited triplet state reacts. Either or both of these reactions may cause cell death. The third mechanism is energy transfer from the excited triplet state to some other molecule, which then initiates a chemical reaction. A molecule which is frequently the receptor of triplet energy is oxygen. Ground state triplet oxygen and triplet state photosensitizer undergo a spin-allowed energy transfer to produce ground singlet state photosensitizer and excited singlet oxygen. This singlet oxygen causes oxidation reactions which kill the cell. The distance the singlet oxygen travels in an *in vivo* or *in vitro* environment is limited by how far it can diffuse during its lifetime. Since singlet oxygen has a lifetime of 5 μs in water, it can travel 20 μm. Therefore, the toxic effect is very localized around the photosensitizer. The first two mechanisms lead to reactions often referred to as Type 1, while the third mechanism produces reactions of Type 2. A schematic of these two reactions is shown in Figure 1. If the photosensitizer dissociates or reacts, the

photosensitizer is destroyed and cannot participate further in the destruction of cells. However, if the cell-killing mechanism is primarily due to localized thermal or energy transfer, then the photosensitizer is returned to the ground state, from which it can absorb another photon. Thus, a single photosensitizer molecule can produce a large number of singlet oxygen molecules. It is generally believed that the Type 2 reaction with oxygen is the dominant reaction in PDT.

To be effective for PDT, the light source must satisfy several requirements. It must emit a suitable wavelength or band of wavelengths for activating the photosensitizer. It should be focusable into a fiber optic for PDT that requires a precise geometry of illumination (e.g., spherical or cylindrical), or for PDT performed in areas that cannot be directly illuminated by the light source (e.g., endoscopic procedures - lung, esophagus, bladder). In addition it should have an output power high enough to ensure that the required light dose can be delivered to the patient in a reasonable time. It also should have a suitable pulsed or continuous wave (cw) characteristic, so that the light interacts effectively with the drug and does not damage healthy tissues or the optic that transmit or reflect the light.

In the case of the last requirement, if the light is emitted as a series of pulses (e.g., pulsed laser source), then the high peak power associated with a single pulse can damage tissue or fiber optic by a number of mechanisms. These mechanisms include ablation, thermal effects and acoustic shock waves. Similarly, if the cw power is too high, then direct damage to the tissue can result such as dehydration and charring. A more complete discussion of various aspects of these

mechanisms is given in the 'Proceedings of "Laser-Tissue Interaction" Conference', SPIE, Vol. 1202 (1990).

In general, average laser powers of a few watts are used, the maximum power being limited by existing sources of high-power lasers that operate at the correct wavelengths. Where pulsed lasers are used, they are usually systems of high pulse repetition rate (1 kHz or higher), with a low output energy/shot (about 1 mJ).

It is the power density (intensity) of the light that determines the effect of the light on the targeted tissue. Power densities of 10-200 mW/cm^2 are typically used for PDT. These are too low to significantly heat up the tissue, but are adequate to activate the photosensitizer, producing the desired effect in a time that is short enough for clinical treatment, this typically being less than 1 hour or so.

By "clinical treatment" we refer to the actual procedure of bringing a patient who has been given the photosensitizing drug into an operating room and delivering the required light dose to the target tissue. As the light dose is calculated using

$$\text{Light Dose } (J/cm^2) = \text{Power Density } (W/cm^2) \times \text{Time (s)},$$

it is clear that if 100 J/cm^2 is needed to activate the drug and produce a "cure," then the time required to do this is determined by the power density available from the light source: e.g., if only 1 mW/cm^2 is available, then 100,000 seconds, or 27 hours 46 minutes, are needed. If 150 mW/cm^2 is available, then only 11 minutes 7 seconds are needed. In terms of patient comfort and physician and operating room time, the high power densities that lead to shorter treatment times are obviously desirable.

These power density requirements also limit the maximum area that can be treated by existing lasers. Typically around 1-4 W of power is available from a laser. The maximum area that can be treated is defined by

$$\text{Area } (cm^2) = \text{Power } (W) \div \text{Power Density } (W/cm^2),$$

which gives an area of 27 cm^2, assuming that 4 W at 150 mW/cm^2 was used. Some procedures require much larger areas than 27 cm^2 to be treated. At present these can only be done with multiple treatments, each treatment step being at a new location. With a higher power light source, larger areas could be treated with a single treatment.

Until now the only light sources that have satisfied all of the PDT criteria given above were large laser systems, such as argon-ion pumped-dye lasers, copper vapor pumped-dye lasers, gold vapor lasers, excimer pumped-dye lasers, and the so-called KTP pumped-dye lasers.

Patented examples of specific lasers being used for PDT may be found in U.S. Patent 4,336,809 to Clark, which discloses a xenon-ion laser, and U.S. Patent 4,614,190 to Stanco et al., which discloses a pulsed-laser photoradiation scheme. Both of these are complex gas-laser-based technologies. Many other practical examples of these and other lasers being used in clinical procedures exist within the scientific and medical literature.

A number of ingenious non-laser sources have also been developed that partially fulfil the requirements, but they are only suitable for treatment of small lesions and are limited in the power they can provide to a fiber system for endoscopic applications.

U.S. Patent 4,757,431 to CROSS discloses the use of off-axis concave spherical reflectors as condensing and collecting optics to couple the optical output of a xenon arc lamp efficiently into a single fiber. U.S. Patent 4,860,172 to Schlager et al. discloses the use of a tapered coupling cone to concentrate the focused optical output of an arc lamp that is mounted in a parabolic reflector, so enabling higher optical powers to be coupled into a fiber.

While the laser has revolutionized PDT, the existing large-frame technology has a number of disadvantages that can be summarized as follows. The devices are physically very large. They have a poor wall-plug efficiency, where this is defined with respect to the electrical power input relative to the laser power output. This results in a requirement for substantial electrical power needs and special main supply outlets rather than the normal house wall-plug socket. They require water or air cooling. They are not very portable. They require periodic realignment by a trained engineer. They require a warm-up time before use and a cool-down time after use. They are very expensive and have substantial maintenance costs. They are limited in the total power they can output in the wavelength range of interest.

Light-emitting diodes (LEDs) are available that operate in the red and near infrared with power outputs up to several milliwatts/LED, they typically being continuous wave (cw), not pulsed. When the LEDs are combined into a suitable array, then power densities up to 200 mW/cm^2 can be obtained. This power density is equivalent to that now being provided by lasers in PDT. Depending on the wavelength required, a variety of possible semiconductor material structures are possible. Examples include AlGaAs and GaInP/AlGaInP, which can be

designed to access wavelengths in the 600-900 nm range by carefully adjusting the parameters used to make the device. One such method is to fine-tune the ratio of the constituents in $Al_xGa_{1-x}As$ to produce the desired results. LEDs at a variety of wavelengths are available from commercial suppliers and are described in company literature, e.g, Hewlett-Packard, Toshiba and Sony.

There is only one previously reported use of this type of technology in PDT. U.S. Patent 4,822,335 to KAWAI et al. reports the use of low-power photodiodes (where these can be laser diodes or LEDs) in a biostimulative form of PDT. The '335 patent describes the use of two distinct wavelength photodiodes (630 nm and 690 nm) that, when used together, activate the photosensitizer HPD (hematoporphyrin derivative) in a synergistic manner to get the desired result. This dual wavelength excitation was believed to access a specific excitation route of the photosensitizer, this being 630 nm excitation to the singlet excited electronic state, followed by the radiationless formation of a triplet excited state, at which point a 690 nm photon would excite the photosensitizer into a higher triplet excited state prior to the usual Type 1 and Type 2 reactions that have been described earlier. At the low power levels the inventors used, they achieved incomplete activation of the drug, and minimal cytotoxicity was observed after 48 hours. They advocate using arrays of photodiodes to treat the target tissues, whereby these arrays would be made up of equal numbers of the two types of photodiode required. The long durations required to kill the target cells with the method advocated by Kawai et al. would not be suitable for any clinical treatment.

It is the intention of the present inventors to demonstrate the effectiveness of higher power LEDs emitting a single wavelength band for PDT, so

demonstrating their ability to overcome all of the problems described above that apply to conventional laser technologies when used for PDT of skin and mucosal tissues and in extracorporeal and intraoperative applications.

Summary of the Invention

The invention comprises high-power LED systems that use a single wavelength band to photoactivate the drug within a clinically acceptable time of an hour or so. The wavelength band is centered around a suitable absorption band of the photosensitizer. The LEDs are configured into arrays that permit PDT treatment of skin and mucosal tissues, and extracorporeal, *in vitro*, and intraoperative applications, e.g., psoriasis, papilloma virus, port wine stains, bone marrow purging, ovarian cancer surgery. None of these applications require the light to be delivered to the target area by a fiber optic system because they can all be directly illuminated by a suitably positioned LED array. The most important requirement for these applications is the ability to perform PDT over the entire area, which can be large, within a period of time that is clinically acceptable for the procedure being performed.

A multiplicity of LEDs can be assembled into a system with their optical power adding together to produce a combined power output that is suitable for PDT over large areas. The maximum power attained is limited only by the number of LEDs that are combined in the particular system. As the LEDs are normally connected in arrays, it is the cumulative power density that is normally measured rather than the power of a single LED; i.e., over an array surface the LEDs are configured to produce the power density needed for the particular treatment.

Using large-area LED arrays would permit scaling of some of the existing PDT procedures which currently are limited by the powers available from conventional laser sources. For example, in a typical dermatological procedure, light intensities of 150 mW/cm^2 are used. At 690 nm the maximum power readily available from a 20 W argon ion-pumped dye laser is 1.5-3.0 W, so limiting the treatment area to 10-20 cm^2. With multiplexed LEDs, areas of thousands of square centimeters can be treated simply by adding more LEDs to the array.

The LEDs can be specifically engineered to produce the desired wavelength output for activating almost any photosensitive drug. Current examples of photosensitizing drugs include PHOTOFRIN® which is activated at around 630 nm, BPD at around 690 nm, and the phthalocyanines and purpurins in the 650-680 nm range. All of these drugs have fairly broad absorption bands, so an ultranarrow laser bandwidth source is not necessary to activate the photosensitizer. It is this broad absorption band that permits LEDs to be used as a replacement for the more conventional laser source, because although LEDs typically have a bandwidth of 20-30 nm, the optical power within this bandwidth is able to interact with the photosensitizer.

LEDs are ideal for PDT as they can be made into compact systems and so can be truly transportable. They have very high wall-plug efficiencies (generally >10% in comparison to the more typical 1% or less of conventional lasers) and so can be powered directly from a standard wall-plug socket rather than a special high power supply. They require little or no air or water cooling. They can be used immediately after being switched on and can be switched off with no major cool-down cycle. LEDs are mass-producible, can be made into a compact integrated

package, and are cheap. They require no maintenance, and so systems assembled from them have little or no maintenance costs. Power feedback and wavelength monitoring can be built into a system to ensure that the optical parameters do not change during the treatment, this being very important if the correct light dose is to be delivered.

From a practical point of view, LEDs have a number of other advantages. They operate at low voltages of about 2 V, so they can be packaged into systems that are powered from a wall socket or a battery pack. These low voltages also simplify compliance with electrical safety and medical requirements: they can be easily carried or wheeled around on a small trolley, or assembled into shapes or sizes appropriate for specialized applications. The monolithic structure of the LEDs makes it simple to design systems that can satisfy the requirements for sterility in a hospital environment. It is possible to connect LEDs together in a variety of ways so that the voltage-current requirements are tailored to the electrical power supply that is available.

Other features and advantages of the invention will be pointed out below, or will become apparent from the drawings, specification and claims that follow.

Description of the Drawings

Figure 1 is a schematic of the Type 1 and Type 2 photochemical reactions of photosensitizers.

Figure 2 is an absorption spectrum of BPD in ethanol, showing the typical broad absorption band in the red, around 690 nm, as well as the extended absorption down into shorter wavelengths. Also, the absorption of oxyhemoglobin is superimposed showing the reduction in oxyhemoglobin absorption as longer wavelengths in the red

are used, i.e., as more light penetrates the tissue to interact with the drug. The y-axis denotes the molar extinction coefficients.

Figure 3 is a typical emission spectrum of a Hewlett-Packard LED centered at 694 nm with a full-width half-maximum (FWHM) bandwidth of 25 nm.

Figure 4 is a block diagram of the overall system concept.

Figure 5 is a block diagram illustrating ways in which LEDs can be connected up so that their optical powers add to give a single broad-area light output with a predictable light intensity: (a) shows overlapping beams from each LED, (b) shows simple rows of LEDs all equally spaced, (c) shows the doubling of power density available from (b) that can be achieved by inserting additional rows of LEDs.

Figure 6 shows the change in the central wavelength of light emission from 690 nm LEDs as the operating temperature of the system is changed.

Figure 7 shows the change in the system temperature of an array of LEDs mounted on a board as the input electrical power is increased.

Figure 8 shows the shift in the central wavelength of the LEDs as the input electrical current is increased.

Figure 9 shows the shift in wavelength of the overall spectrum of light emission from the LEDs and the associated increase in light intensity as the electrical current input is increased.

Figure 10 shows the change in light intensity output when electrical current input is varied.

Figure 11 shows the change in power density with increasing distance from a 3.5 cm x 3.5 cm array.

Figure 12 shows the uniformity of light intensity from a 3.5 cm x 3.5 cm area array.

Figure 13 shows a typical geometry of LEDs incorporating a lens (Hewlett-Packard data sheet, type T1).

Figure 14 shows how the LEDs can be incorporated into specific geometries for treating an entire person, part of that person, or a sample area of the tissue or cells of interest.

Figure 15 shows results of the *in vitro* cytotoxic dose response (MTT assay), with the percent killed or impaired being compared to the controls (0.0 μg BPD), for a LED array and the argon ion-pumped dye laser (APDL).

Figure 16 is an *in vivo* equivalency study showing the percentage of animals tumor-free from day 0 and day 20 after exposure to the APDL and the LED array; a fixed light dose of 150 J/cm^2 and a BPD-MA drug dose of 2 mg/kg were used for all the animals.

Modes of Carrying Out the Invention/Description of Preferred Embodiments

The LEDs will be chosen such that they efficiently and specifically activate the desired photosensitizer. For example, in the case of PHOTOFRIN*, wavelengths in the range of 630 ± 30 nm would be suitable. In the case of BPD, wavelengths in the range of 690 ± 30 nm would be suitable. An excitation wavelength can be generalized in this way and still be effective in activating the photosensitizers because their absorption bands are fairly broad, those for BPD being shown in Figure 2. The BPD absorption band at 690 nm can be compared to that of a typical 690 nm LED source that is shown in Figure 3. These LEDs are based on a transparent substrate AlGaAs material, and are obtained from Hewlett-Packard (product type T1 TS AlGaAs 690 nm). The FWHM of the LED spectrum is about 25 nm,

this being similar to that of the 690 nm BPD absorption band.

Where there is some choice between absorption bands that can be accessed by a LED, the one used will be determined by the treatment being performed. In general, maximum penetration of light into the tissue to be treated is required, and this necessitates the use of longer wavelengths in the red and near infrared. However, under circumstances where there is a specific requirement to treat to a well-defined depth, then a shorter wavelength of light can be used to activate the drug, thus limiting the depth of light penetration.

Various types of photosensitizers can be used in conjunction with this technology, including PHOTOFRIN* and BPD, which can be obtained from Quadra Logic Technologies, Inc., Vancouver, B.C., Canada.

Figure 4 shows some of the desired features in the LED system. Power source 1 can be either an AC-to-DC supply or a battery source. The LEDs can be designed into the system in such a way that they can be disposed of either with the system, or as a disposable component that is replaced by a new plug-in LED module 2. Typically, this type of LED has a life of over 10,000 hours, although this is a strong function of the electrical current and cooling. If another wavelength is needed to activate the particular photosensitizer in use, then another LED module 2 with a suitable wavelength output can be connected.

Feedback loop 3 monitors the LED power output via photodiode detectors (output light power is sampled) and via the electrical current being supplied to the LED. The signal detected by either of these methods can be used to stabilize the LED light power output by comparing it to previously set reference levels and adjusting the

electrical power input to compensate for any changes detected.

A second feedback loop 4 can be connected to an external dosimetry system that monitors either the light actually delivered to the tissue or the real-time phototherapeutic effect at the target 5, cutting off the light output when the desired light dose or effect has been achieved.

The LED light output can be cut off during or after treatment simply by cutting the electrical power. This cannot be done with the existing mainframe lasers; a shutter system must be used. A low power output can be easily obtained for alignment purposes prior to treatment either by having a preset low electrical power input or a variable input power supply.

Other features that are incorporated into existing PDT light sources that aid the user of the device can also be used with LEDs. Examples of these include built-in timers used to calculate the light dose delivered, green LEDs or backlit LCD displays that can be read even while wearing laser safety goggles (the goggles filter out the red display panel figures).

While it is preferred that the output should be cw, this is not an essential requirement, and a LED array that is pulsed will work provided that its pulse characteristics are suitable for activating the drug and its average power is high enough.

In existing clinical trials the areas that can be treated are limited by the power available from the laser. With LEDs the area that can be treated is limited only by the number of LEDs that can be connected together in the system and the power supply available. Figure 5 illustrates how the LEDs are connected together to form an array with the output of all the elements adding together. A uniform power density is obtained in the

center of the array, with the power density dropping off as the edges of the array are reached and passed, this being shown in more detail in Figure 13.

The packing density of the LEDs in the array is also critical in determining the power density that can be obtained from it. This can be understood very easily if one considers that if each LED has a fixed power output of, for example, 5 mW, the number of LEDs/cm^2 multiplied by this power output will determine the power density in mW/cm^2. As the number of LEDs/unit area is increased, the maximum power density that can be obtained from the array increases proportionately; a method by which this is done is shown in Figures 5(b) and 5(c). The physical size of each LED in its mounting and the problems of removing the heat from the LED array ultimately limit the maximum packing density that can be used. In Figure 5(a), 11 refers to the circuit board of PCB to which the LEDs are connected, 12 refers to the individual LEDs, and 13 is the region in which their beams overlap.

There are a number of factors that have to be carefully considered in setting the power and wavelength characteristics of the LEDs. Figure 6 shows the shift in the central wavelength of the LEDs as the temperature changes, this being about 0.25 nm/°C for these LEDs. This means that careful control of the LEDs' temperature can fine-tune the central wavelength of the light output.

In practice, a temperature change is also seen as the electrical power input to the LEDs is changed, assuming a fixed rate of cooling. Figure 7 shows how the LED temperature changed as the electrical power input was increased under these conditions. This can also be observed in Figure 8, which shows the wavelength shift with input electrical current to the LEDs. The overall effect of increasing the input electrical current can be

seen in Figure 9. As the electrical current is increased, the power output of the LEDs increases, and the emitted wavelength band shifts to longer wavelengths.

Figure 10 shows the change in power density measured 2 cm in front of an array as the electrical current into the array is increased. This demonstrates that the optical power in the range of 0-200 mW/cm^2 can readily be obtained with a suitably designed array.

Figures 5 to 10 illustrate that with judicious use of cooling (for example, using forced air or water) and careful selection of the operating electrical current for a fixed LED packing density, the central wavelength of the wavelength band from the LEDs can be set to coincide with an absorption peak of a photosensitizer while operating at a predetermined power output. In general, the above parameters would be preset during manufacture for the particular device in question, so that the hospital operator would not have to make adjustments to the device once it was installed.

The change in power density as one goes farther away from an array is shown in Figure 11, where this array is 3.5 cm x 3.5 cm. This shows that the light intensity drops fairly rapidly as the distance increases. Figure 12 demonstrates the good light power density uniformity that is obtained with such arrays, where the center of the array of Figure 11 is located at x=5 cm and y=6.5 cm. All the measurements of light power density seen in these figures are taken using a small diameter spherical diffuser fiber (from Quadra Logic Technologies, Inc.), where the diffusing sphere at the distal end samples the light density at a given point above the LED array. The portion of light captured by the fiber is then transmitted to the proximal end where a calibrated photodiode that takes into account the optical

transmission of the fiber detector system is used to determine the light density at that point.

Figure 13 shows a typical LED (the one used to collect the data for this patent) that is available from Hewlett-Packard. This design incorporates a lens so that the light is emitted as a beam with a solid angle of about 0.6 steradians.

Several arrays of LEDs can be connected together permitting a single area equal to their combined areas to be treated. Figures 14a through 14c show examples of a number of geometries that the LEDs can be configured into, some of which take advantage of this ability to combine a number of panels together. Figure 14a illustrates the use of LEDs mounted to form an array on a flexible or folded circuit board. The numbering here denotes: the LEDs 21 mounted on the circuit boards 22, and the emitted light 23 incident on the target 24. As is shown here, this could be used to treat localized tissue areas with curved surfaces, e.g., the arm or leg, or areas opened up for surgery.

Figure 14b shows large area panels connected to form a box, or "entire body phototherapy system." This system could be used to treat patients with extensive psoriasis. In FIG. 14b two panels 27 and 28 carry light emitting diodes which emit light 26 and 26, respectively, onto target 29. If required front and back panels may include light emitting diodes too.

Figure 14c shows the use of a simple panel of LEDs for a localized cutaneous treatment of tissue, e.g., basal cell carcinoma or port wine stains. Light emitting diodes 31 mounted on panel 32 emit light 33 onto the treatment area of target 34

The way in which the LED arrays are connected to the control unit will depend on the clinical operation. One method is to have an array of LEDs,

designed to activate a specific drug, attached via an umbilical line to the control unit. The umbilical line will carry power to the LEDs as well as relaying information on their operating optical power output and temperature, etc. back to the control unit. This geometry is useful where the maximum flexibility is needed in positioning the array because of space constrains. Note also, that it is possible to disconnect an array and its umbilical line from the control unit, and to substitute it with a second array that has the required wavelength output to activate a different drug or to achieve greater or less penetration into tissue. Where space is less of a problem the LED arrays can be connected directly to the control unit, and again different LED panels can be substituted in as different wavelengths are needed to activate different drugs, or achieve greater or less penetration of light into tissue.

The following are examples of the use of this invention that demonstrate the effectiveness of high-power LEDs when used at an appropriate wavelength with a suitable photosensitizer. In these example the photosensitizer is BPD-MA (monacid form of BPD) activated by LEDs operating at wavelengths centered around 690 nm.

Example 1

An *in vitro* study on BPD-MA-treated P815 mouse mastocytoma cells compared the cytotoxic effects of 690 nm laser light from an APDL and light from a LED array with a 25-nm bandwidth centered at 691 nm. The P815 cells were incubated for 1 hour at 37°C in the dark with various doses of liposome-formulated BPD-MA. After being washed, the cells were placed in duplicate 96-well tissue culture microtiter plates at 1×10^5 cell/well.

Plates were exposed to 2 mW/cm^2 of light for 30 minutes from either the laser or the LED array, this

corresponding to a light dose of 3.6 J/cm^2. Cytotoxicity was assessed 24 hours later using the MTT assay. The results are shown in Figure 15.

At a drug dose of 50 ng/ml, the cytotoxicity achieved by both light sources was 100%, indicating that with high doses of light LED sources are as efficient as laser sources at effecting maximum cell kill or impairment in photosensitized cell populations in the 30-minute exposure time used here.

Example 2

Male DBA/2 CR mice were implanted with M1-S (rhabdomyosarcoma) cells 10 to 12 days prior to the experiment and depilated 6-7 days later. Animals carrying 5-mm diameter tumors were dosed i.v. with a single dose of liposome-formulated BPD-MA (2.0 mg/kg). The mice were rested for 3 hours in the dark before exposure to light.

Irradiation was carried out using either an APDL tuned to 690 nm, or an LED array with a 25-nm bandwidth centered at 696 nm. A circular area around the tumor site, 1.0 cm in diameter, was exposed to light at a power density of 110 mW/cm^2. Animals were observed for tumor recurrence over a 20-day period post-exposure. The anti-tumor efficacy data is presented in Figure 16: At day 7, 100% of the mice exposed to LED light were tumor free; at day 14, 40% were tumor free; at day 20, 20% were tumor free. These curves show that a significant anti-tumor effect was obtained *in vivo* with the LED array and the laser under identical treatment regimes.

Claims

1. A system for activating a photosensitizer for *in vivo* or *in vitro* PDT, comprising at least one array of light-emitting diodes and first control means for operating said light-emitting diodes at a predetermined power level and duration to activate said photosensitizer.

2. A system according to claim 1, wherein said array comprising an array of selectively controllable light-emitting diodes and said first control means including means for selectively operating said light-emitting diodes at a predetermined power level and duration to activate said photosensitizer.

3. A system according to claim 1, further including a plurality of arrays of light emitting diodes, each said array emitting light of different wavelength to match the absorption spectrum of the selected photosensitizer, and means for exchangeably mounting a selected one of said arrays of light emitting diodes, said first control means including means for activating said selected array of light-emitting diodes for a desired time period and intensity to activate said photosensitizer for the required tissue penetration.

4. A system according to claim 2, said array of light-emitting diodes including diodes emitting light of different wavelength, said first control means including means for activating light-emitting diodes of a desired wavelength to match the absorption band of the photosensitizer that is being activated, and the required tissue penetration.

5. A system according to claim 1, wherein said first control means operates said light-emitting diodes selectively in a pulsed mode or a continuous mode.

6. A system according to claim 1, wherein a single band of wavelengths is used to activate the photosensitizer.

7. A system according to claim 1, further including a plurality of arrays of light emitting diodes, said arrays emitting light of different wavelength to match the absorption spectrum of the selected photosensitizer, and a plurality of means for exchangeably mounting selected ones of said arrays of light emitting diodes,
said first control means including means for activating said selected arrays of light-emitting diodes for a desired time period and intensity to activate said photosensitizer for the required tissue penetration.

8. A system according to claim 7, wherein said first control means controls a plurality of said arrays of light-emitting diodes such that said controlled arrays of light-emitting diodes produce high intensities suitable for PDT.

9. A system according to claim 1, wherein said arrays of light-emitting diodes are mounted on a flexible circuit board that permits a uniform light dose to be delivered to non-flat surfaces.

10. A system according to claim 1, wherein said arrays of light-emitting diodes are mounted on a

curved circuit board that permits a uniform light dose to be delivered to non-flat surfaces.

11. A system according to claim 1, further including a photodetector for monitoring the power density emitted by said array of light emitting diodes; said photodetector providing an output signal to said first control means;
said first control means including second control means for controlling said array of light emitting diodes in response to said output signal of said photodetector to keep the power density stable by compensating for any fluctuations in the output value by increasing or decreasing the electrical current supplied to the LEDs.

12. A system according to claim 1, wherein said first control means further including input means for receiving an external control signal,
said external control signal providing control information for controlling light density and duration defining the light dose to be delivered to said tissue, and information for terminating said light dose in response to a detected system failure.

13. A system according to claim 1, wherein said first control means including third control means for controlling the output power density, wavelength and temperature of said light-emitting diodes thereby fine-tuning said light-emitting diodes to the optimum levels for activating a given photosensitizer.

14. A system according to claim 1, wherein the photosensitizer used is BPD.

15. A system according to claim 1, wherein the photosensitizer used is PHOTOFRIN®.

16. A method of *in vivo* activation of an administered photosensitizing compound, which method comprises the step of irradiating tissues of interest with light from an LED system, said tissues having accumulated some of said photosensitizer prior to said irradiation.

17. A method of activating a photosensitizing compound, which method comprises administering a photosensitizer and subsequently irradiating an extracorporeal sample with light from an LED system.

18. The method of claim 16, wherein the photosensitizer is PHOTOFRIN®.

19. The method of claim 16, wherein the photosensitizer is BPD.

20. The method of claim 17, wherein the photosensitizer is PHOTOFRIN®.

21. The method of claim 17, wherein the photosensitizer is BPD.

AMENDED CLAIMS

[received by the International Bureau on 3 August 1993 (03.08.93) ; original claims 1-21 replaced by amended claims 1-24 (6 pages)]

1. An apparatus for activating a photosensitizing drug during photodynamic therapy at a single wavelength band, the photosensitizing drug having an active-wavelength absorption band, the active wavelength absorption band encompassing the single wavelength band, comprising:

an array of light-emitting photodiodes configured to have a generally uniform power density between 110 and 250 mW/cm^2 over an area of at least 20 cm^2, each one photodiode outputting light within a common wavelength band centered at a predetermined wavelength, the common wavelength band defining said single wavelength band and spanning approximately 20 to 30 nm;

means for stabilizing the power output of the array; and

means for operating said array for an automatically determined exposure time; and

wherein said area is exposed to the light during said exposure time, the light activating the photosensitizing drug.

2. The apparatus of claim 1 in which the common wavelength band is centered at approximately 690 nm.

3. The apparatus of claim 1 in which the common wavelength band is centered at approximately 630 nm.

4. The apparatus of claim 1 in which the generally uniform power density between 110 and 250 mW/cm^2 is achieved by supplying a current signal to said each one photodiode ranging between approximately 30 and 85 mA.

5. The apparatus of claim 1, in which the stabilizing means comprises:

a photodetector for monitoring output light power of the array of light-emitting diodes, the photodetector generating an output signal based upon to the monitored output light power; and

means for adjusting electrical power input to the

array of light-emitting diodes in response to fluctuations in the photodetector output signal.

6. The apparatus of claim 1, in which the exposure time is approximately 30 minutes or less.

7. The apparatus of claim 1, in which the operating means comprises:

means for monitoring the light dose delivered to said area; and

means for discontinuing output from the array of light-emitting diodes when a desired dose has been delivered to said area.

8. The apparatus of claim 1, in which the operating means comprises:

means for monitoring therapeutic effect at said area; and

means for discontinuing output from the array of light-emitting diodes when a desired therapeutic effect has been achieved.

9. The apparatus of claim 1, wherein the array of light-emitting diodes is mounted on a flexible circuit board shaped to enable delivery of a uniform dose of light to a non-flat surface.

10. The apparatus of claim 1, wherein the array of light-emitting diodes is mounted on a curved circuit board shaped to enable delivery of a uniform dose of light to a non-flat surface.

11. The apparatus of claim 1 in which the operating means controls the light-emitting diodes to operate in either one of a pulsed mode or continuous mode.

12. The apparatus of claim 1, wherein the photosensitizing drug is BPD.

13. The apparatus of claim 1, wherein the photosensitizing drug is PHOTOFRIN.

14. An apparatus for activating a photosensitizing drug during photodynamic therapy at a single wavelength band, the photosensitizing drug having an active-wavelength absorption band, the active wavelength absorption band encompassing the single wavelength band, comprising:

an array of light-emitting photodiodes configured to have a generally uniform power density between 10 and 250 mW/cm^2 over an area of at least 20 cm^2, each one photodiode outputting light within a common wavelength band centered at a predetermined wavelength, the common wavelength band defining said single wavelength band and spanning approximately 20 to 30 nm;

means for stabilizing the power output of the array; and

means for operating said array for an automatically determined exposure time; and

wherein said area is exposed to the light during said exposure time, the light activating the photosensitizing drug.

15. The apparatus of claim 14 in which the common wavelength band is centered at approximately 690 nm.

16. The apparatus of claim 14 in which the common wavelength band is centered at approximately 630 nm.

17. The apparatus of claim 14, in which the stabilizing means comprises:

a photodetector for monitoring output light power of the array of light-emitting diodes, the photodetector generating an output signal based upon to the monitored output light power; and

means for adjusting electrical power input to the array of light-emitting diodes in response to fluctuations in the photodetector output signal.

18. The apparatus of claim 14, in which the operating means comprises:

means for monitoring the light dose delivered to said area; and

means for discontinuing output from the array of light-emitting diodes when a desired dose has been delivered to said area.

19. The apparatus of claim 14, in which the operating means comprises:

means for monitoring therapeutic effect at said area; and

means for discontinuing output from the array of light-emitting diodes when a desired therapeutic effect has been achieved.

20. An apparatus for activating a photosensitizing drug during photodynamic therapy at a single wavelength band, the photosensitizing drug having an active-wavelength absorption band, the active wavelength absorption band encompassing the single wavelength band, comprising:

an array of light-emitting photodiodes configured to have a generally uniform power density between 10 and 250 mW/cm^2 over an area of at least 20 cm^2, each one photodiode outputting light within a common wavelength band centered at a predetermined wavelength, the common wavelength band defining said single wavelength band and spanning approximately 20 to 30 nm;

means for stabilizing the power output of the array; and

means for automatically discontinuing light output when one of either a monitored output light dosage or a monitored therapeutic effect is achieved at said area; and

wherein said area is exposed to the light during said exposure time, the light activating the photosensitizing drug to achieve the desired light dosage or therapeutic effect within approximately 30 minutes.

21. The apparatus of claim 20 in which the common wavelength band is centered at approximately 690 nm.

22. The apparatus of claim 20 in which the common wavelength band is centered at approximately 630 nm.

23. The apparatus of claim 20, in which the stabilizing means comprises:

a photodetector for monitoring output light power of the array of light-emitting diodes, the photodetector generating an output signal based upon to the monitored output light power; and

means for adjusting electrical power input to the array of light-emitting diodes in response to fluctuations in the photodetector output signal.

24. A method for activating a photosensitizing drug at a treatment area during photodynamic therapy at a single wavelength band, the photosensitizing drug having an active-wavelength absorption band, the active wavelength absorption band encompassing the single wavelength band, the method comprising the steps of:

outputting light from an array of photodiodes configured to have a generally uniform power density between 10 and 250 mW/cm^2 over a treatment area of at least 20 cm^2, each one photodiode outputting light within a common wavelength band centered at a predetermined wavelength, the common wavelength band defining said single wavelength band and spanning approximately 20 to 30 nm, the light activating the photosensitizing drug to photodynamically treat the area exposed;

monitoring output light power of the array;

adjusting power input to the array in response to fluctuations in the monitored light power;

determining when one of a desired light dosage or desired therapeutic effect has been achieved;

automatically discontinuing light output upon determining said one of said desired dosage or effect has

been achieved; and

wherein the output light power is sufficient to achieve the desired light dosage or therapeutic effect within approximately 30 minutes.

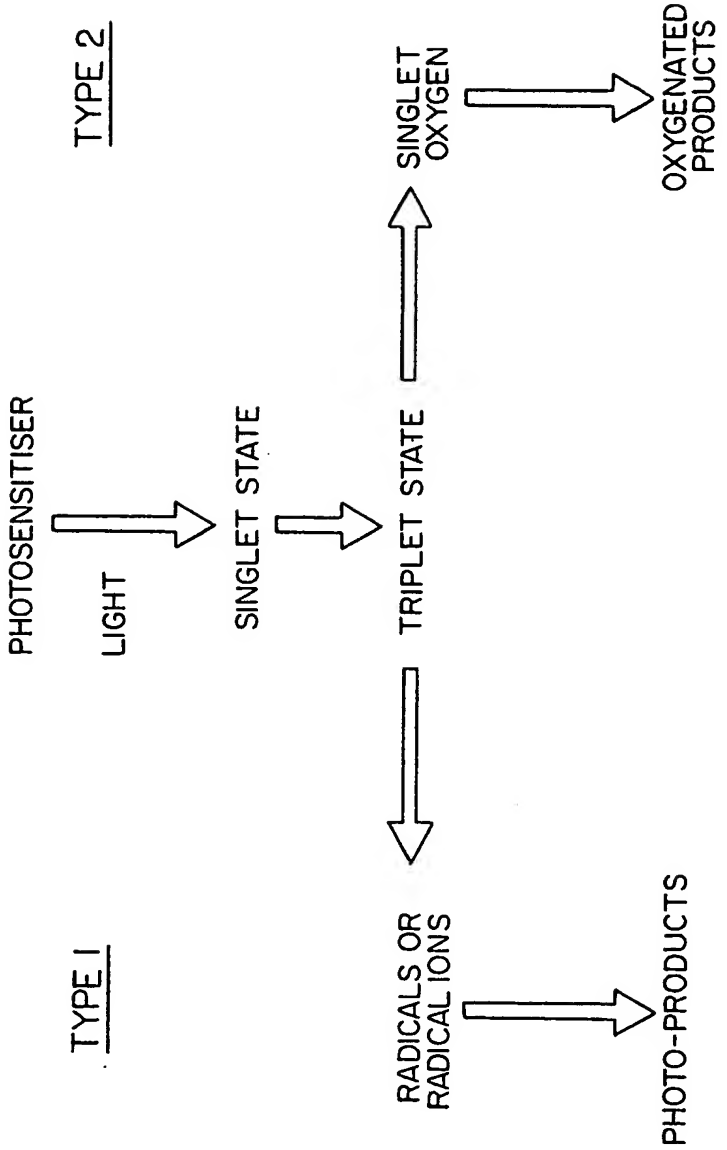
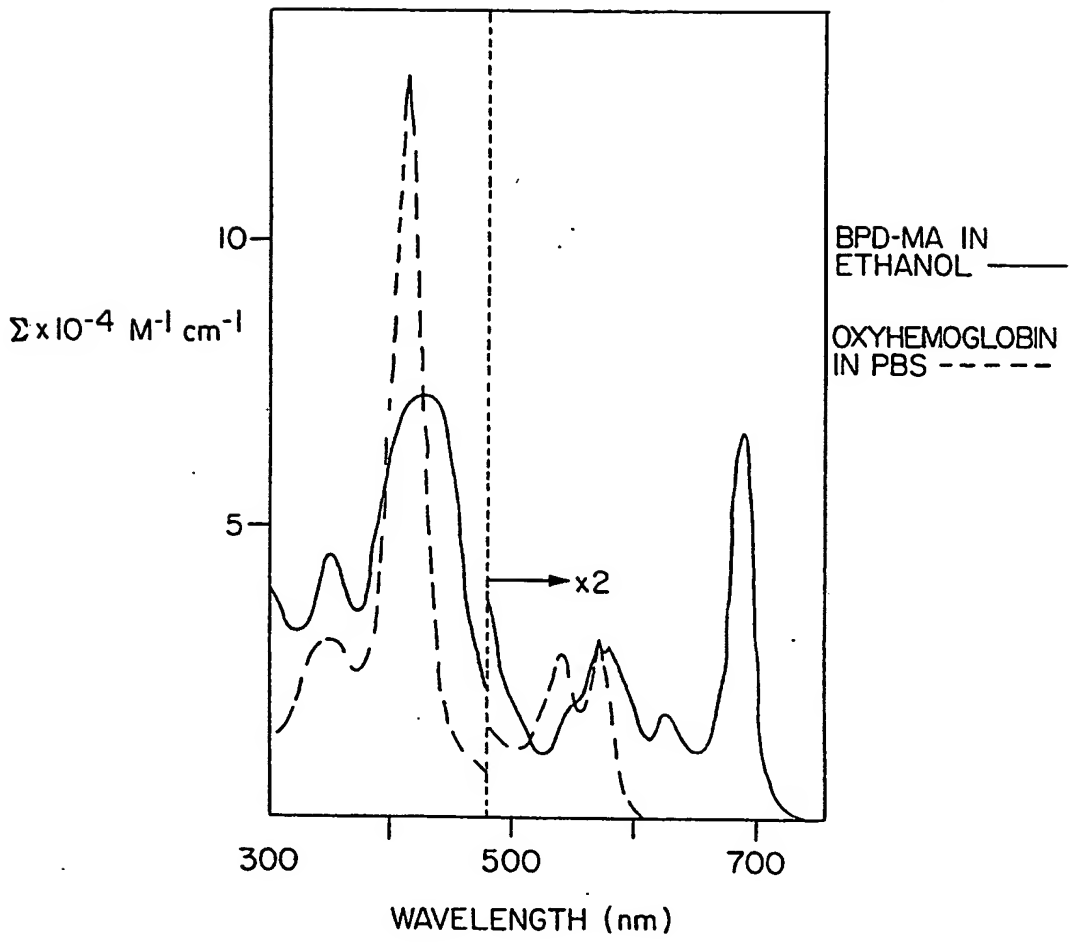

TYPE 2
PHOTOSENSITISER
LIGHT
SINGLET STATE
TRIPLET STATE
SINGLET OXYGEN
OXYGENATED PRODUCTS
TYPE 1
RADICALS OR RADICAL IONS
PHOTO-PRODUCTS

FIG. 1

FIG. 2


10
5
Σ x 10-4 M-1 cm-1
x2
300
500
700
WAVELENGTH (nm)
BPD-MA IN ETHANOL
OXYHEMOGLOBIN IN PBS

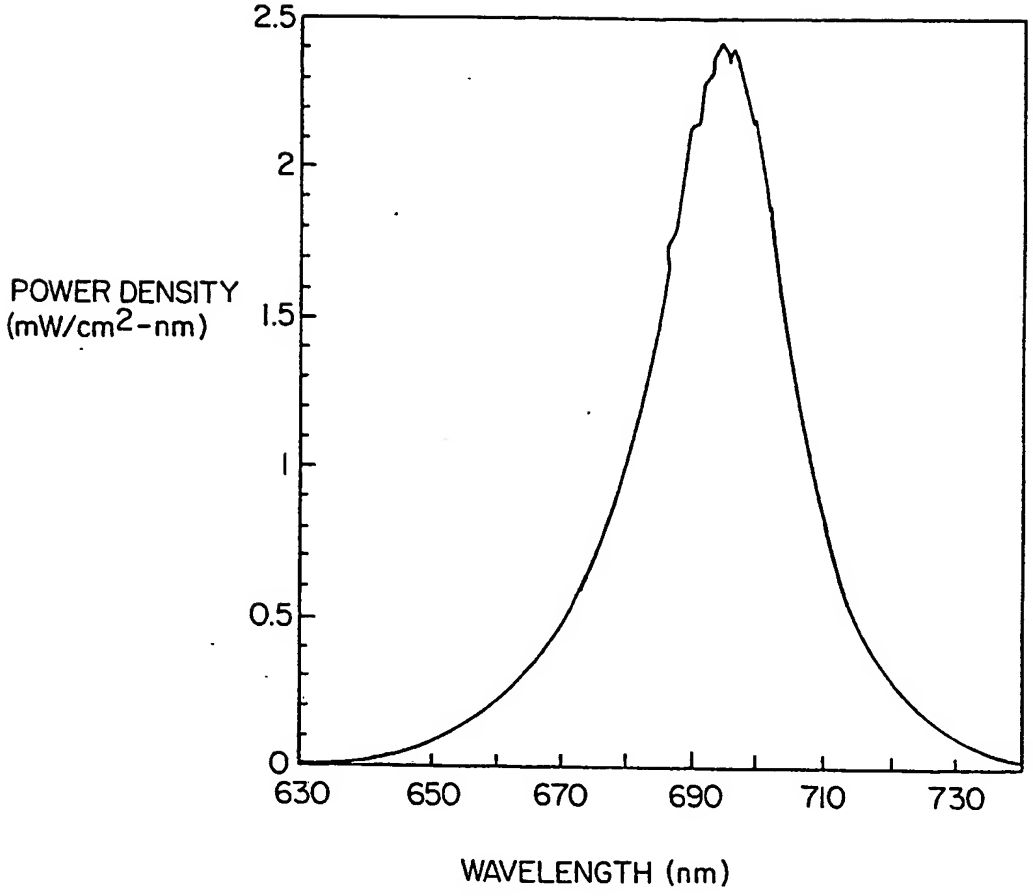

2.5
2
1.5
1
0.5
0
POWER DENSITY
(mW/cm2-nm)
630
650
670
690
710
730
WAVELENGTH (nm)

FIG. 3

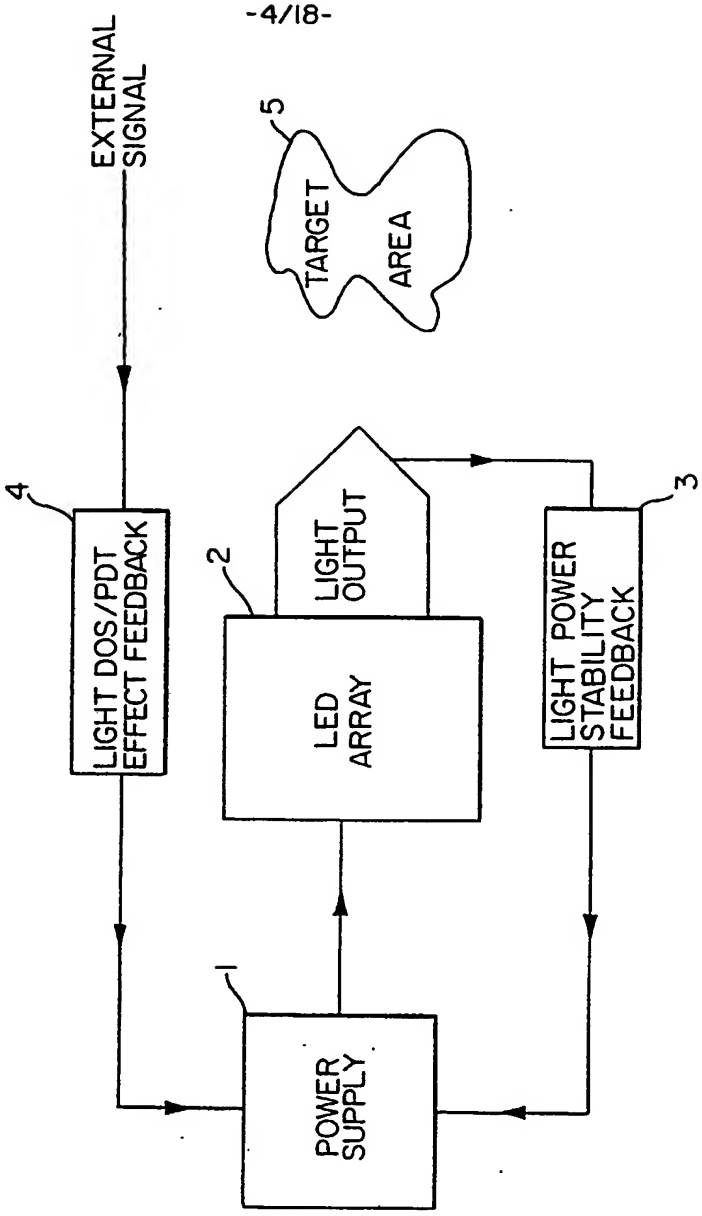

EXTERNAL SIGNAL
5
TARGET AREA
4
LIGHT DOS/PDT EFFECT FEEDBACK
2
LIGHT OUTPUT
LED ARRAY
3
LIGHT POWER STABILITY FEEDBACK
1
POWER SUPPLY

FIG. 4

FIG.5A

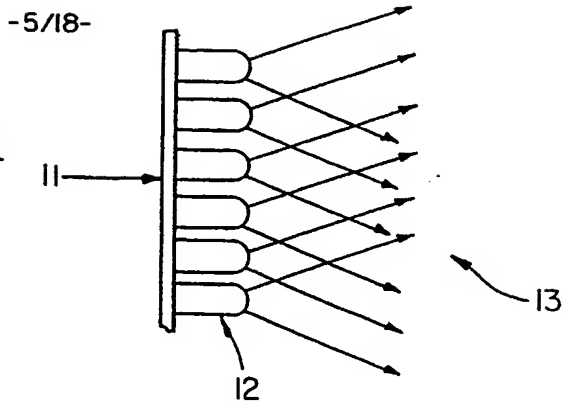


FIG.5B

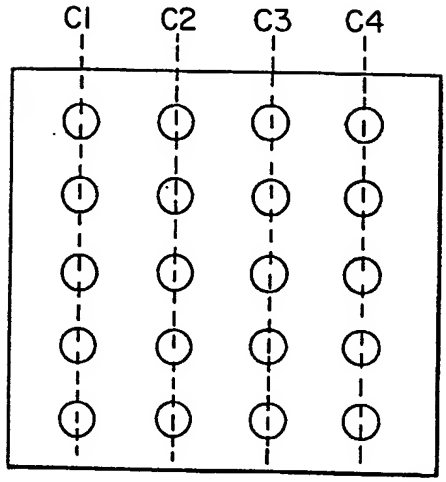
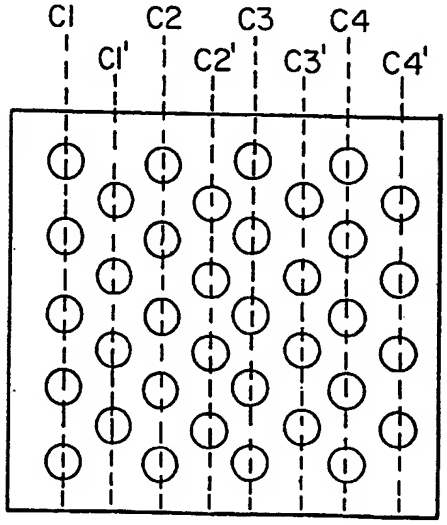


FIG.5C



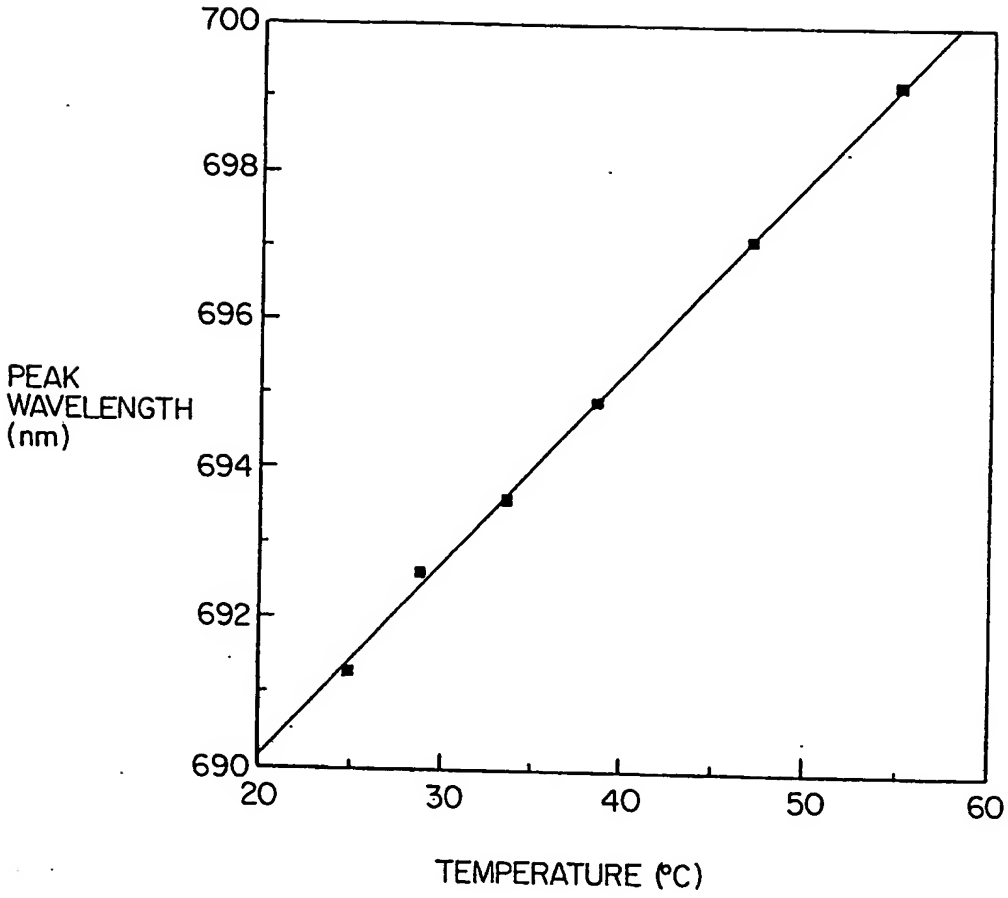

700
698
696
694
692
690
PEAK
WAVELENGTH
(nm)
20
30
40
50
60
TEMPERATURE (°C)

FIG. 6

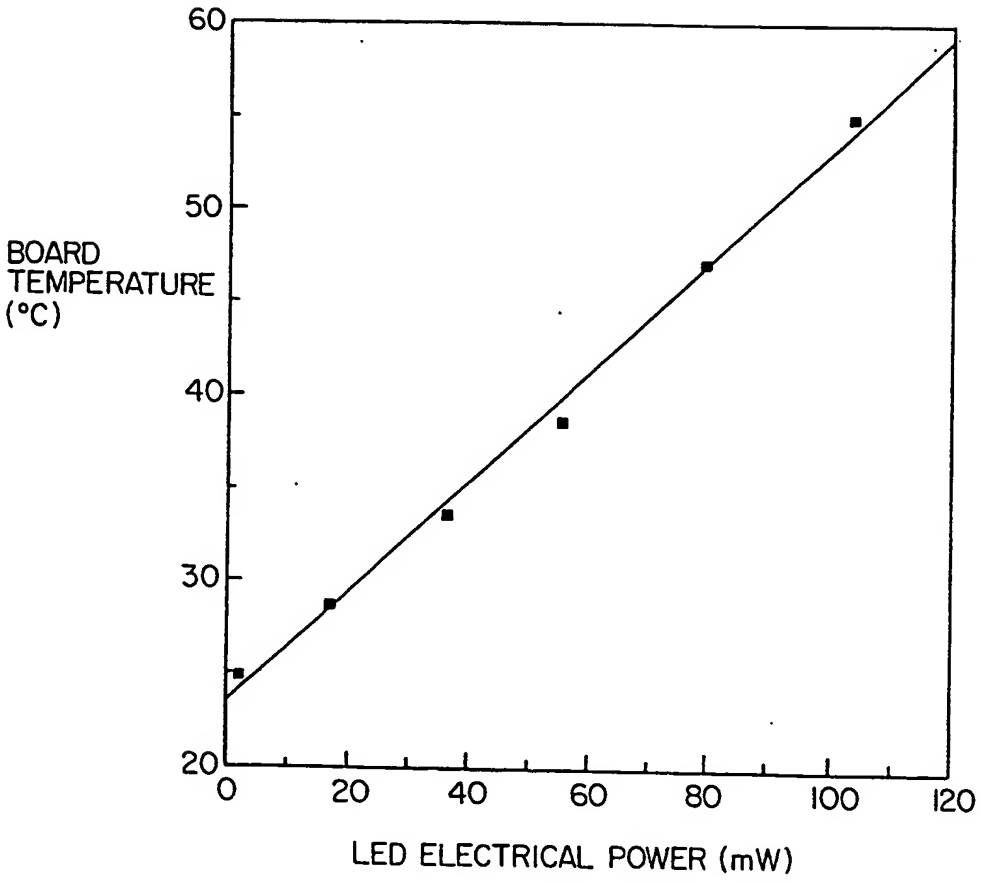
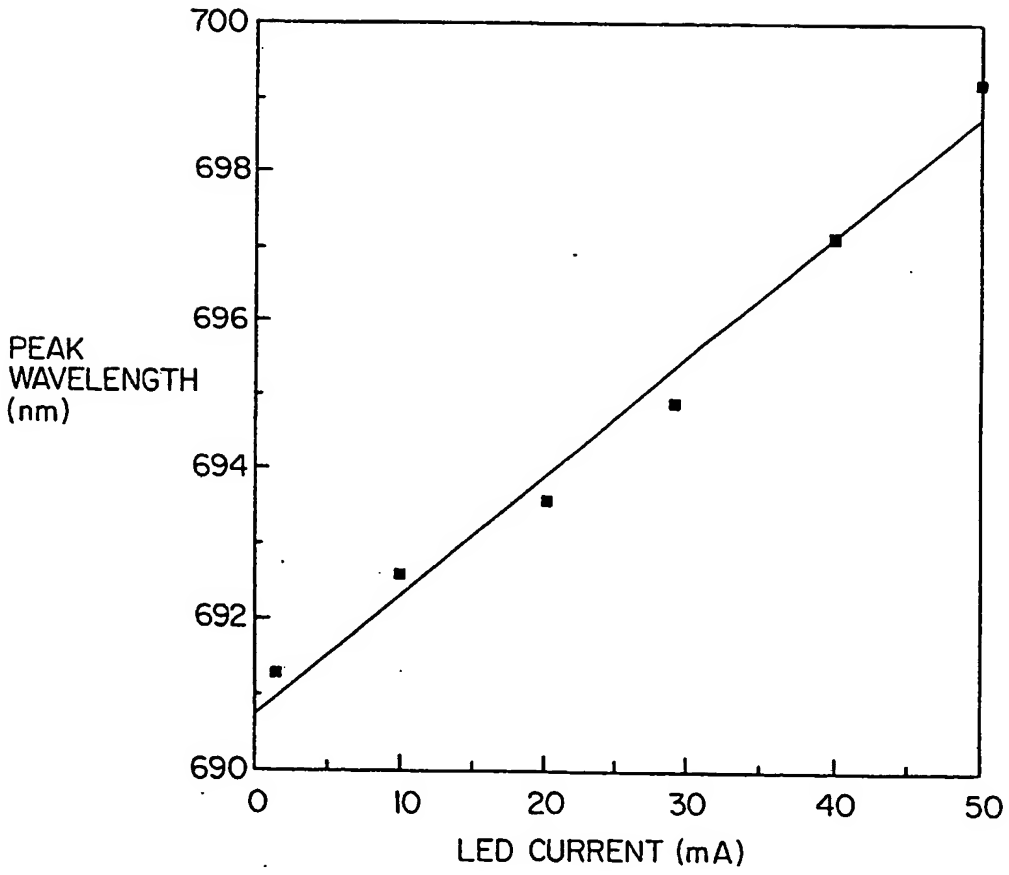

60
50
40
30
20
BOARD
TEMPERATURE
(°C)
0
20
40
60
80
100
120
LED ELECTRICAL POWER (mW)

FIG. 7

FIG. 8


700
698
696
694
692
690
PEAK
WAVELENGTH
(nm)
0
10
20
30
40
50
LED CURRENT (mA)

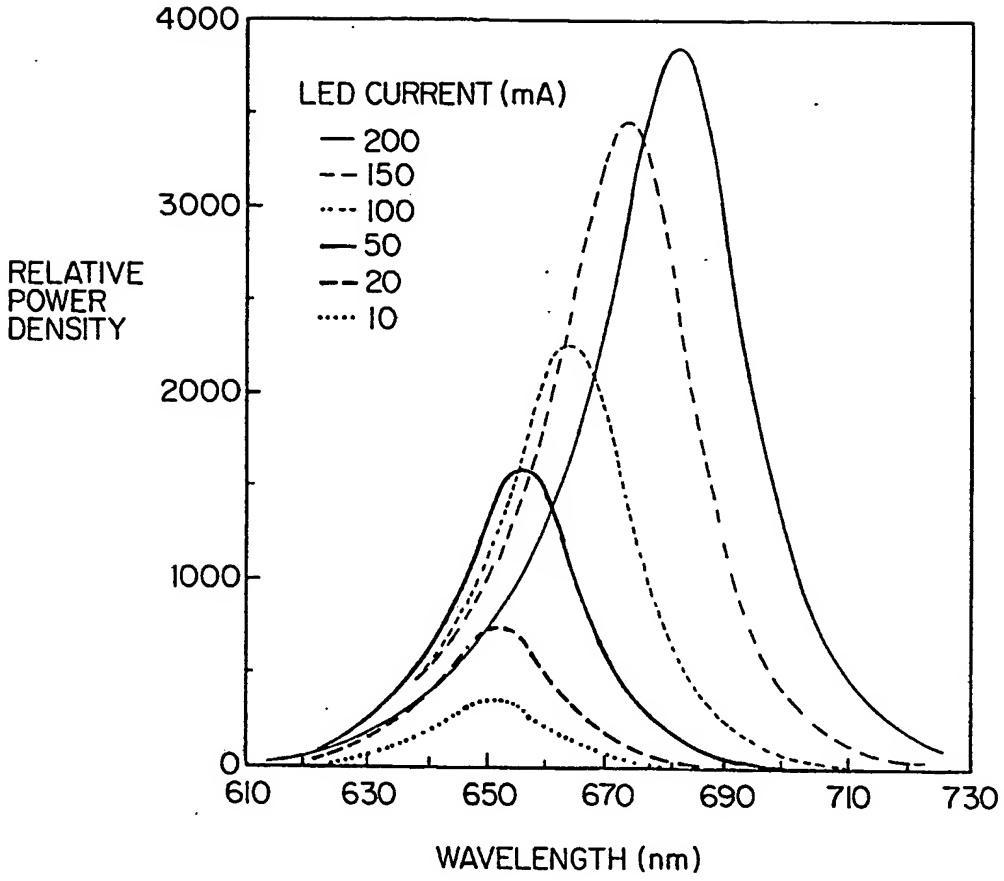

4000
3000
2000
1000
0
RELATIVE POWER DENSITY
LED CURRENT (mA)
200
150
100
50
20
10
610
630
650
670
690
710
730
WAVELENGTH (nm)

FIG. 9

FIG. 10

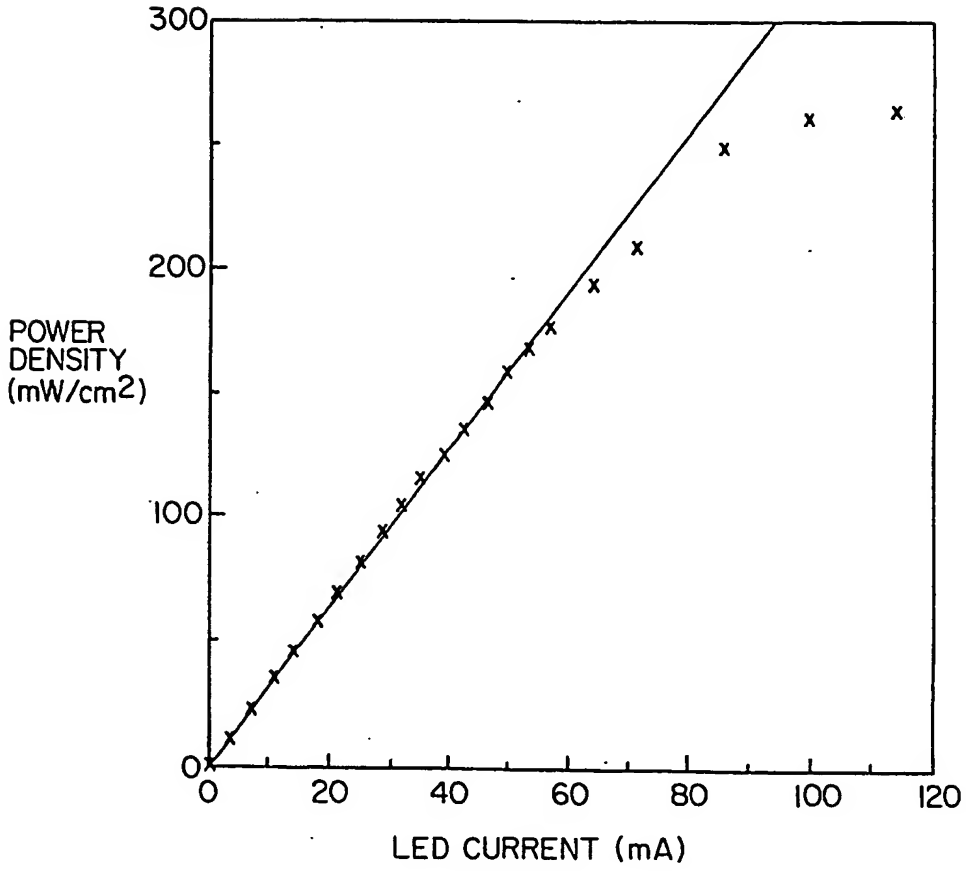
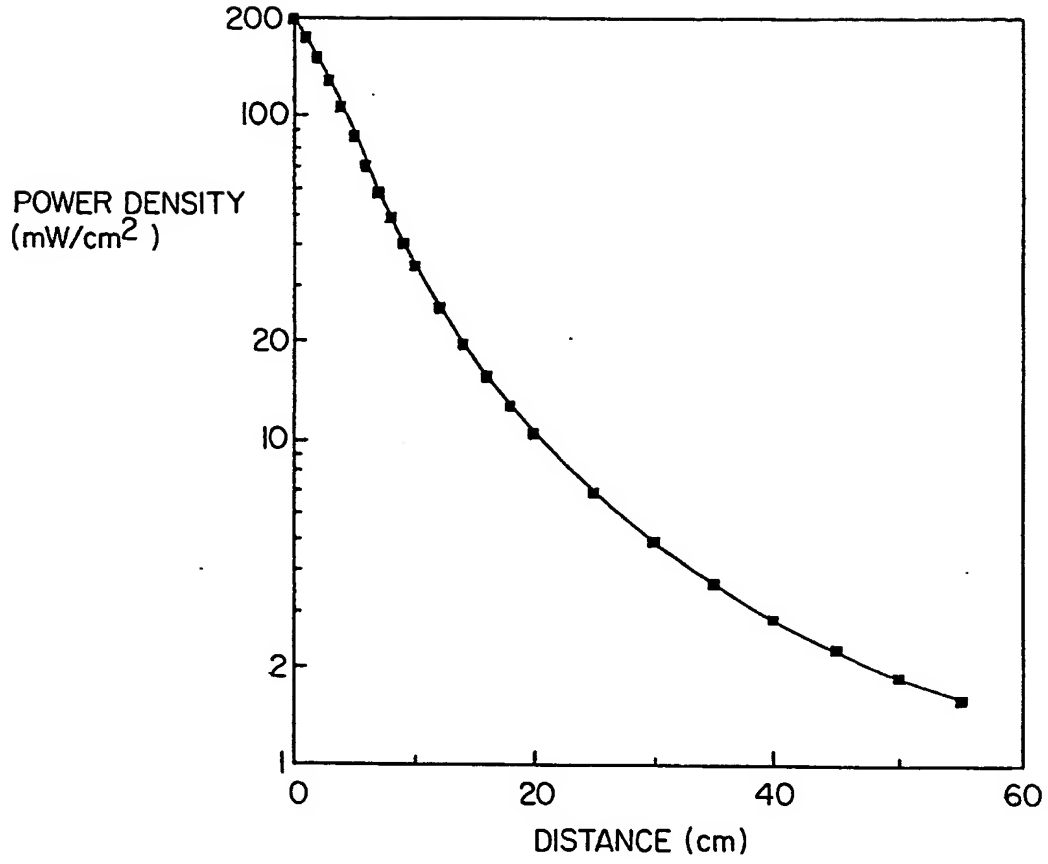

300
200
100
0
POWER
DENSITY
(mW/cm2)
0
20
40
60
80
100
120
LED CURRENT (mA)

FIG. 11



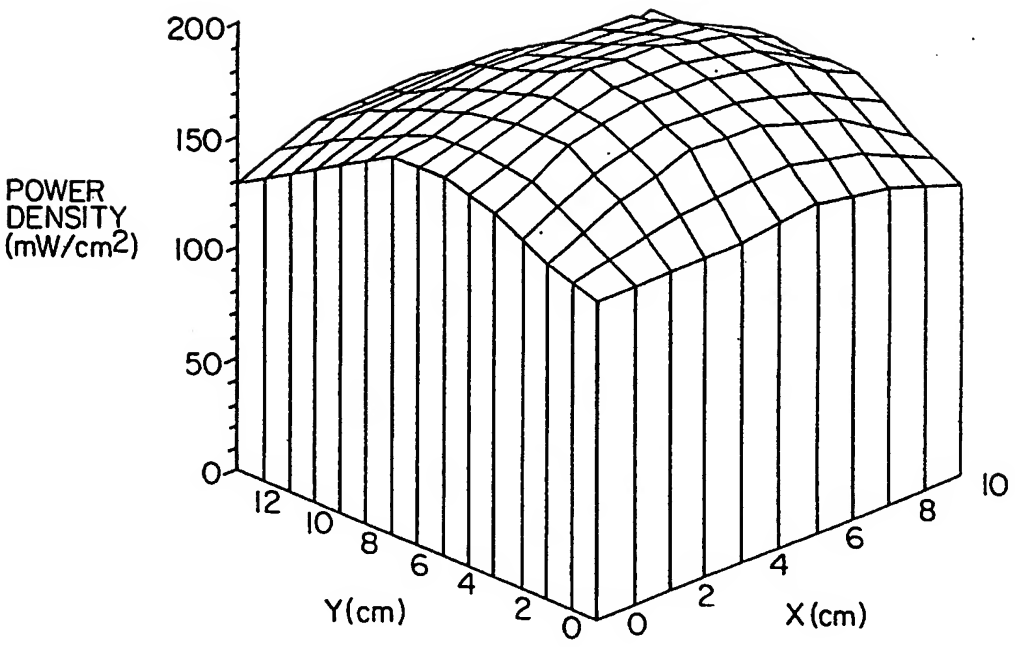

POWER
DENSITY
(mW/cm2)
200
150
100
50
0
12
10
8
6
4
2
0
Y(cm)
0
2
4
6
8
10
X(cm)

FIG. 12

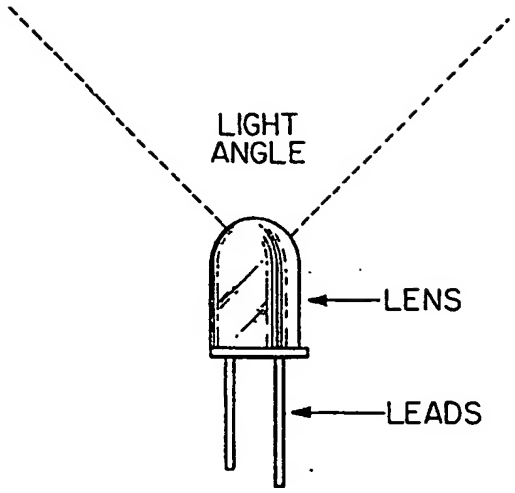


FIG. 13A

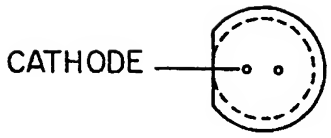


FIG. 13B

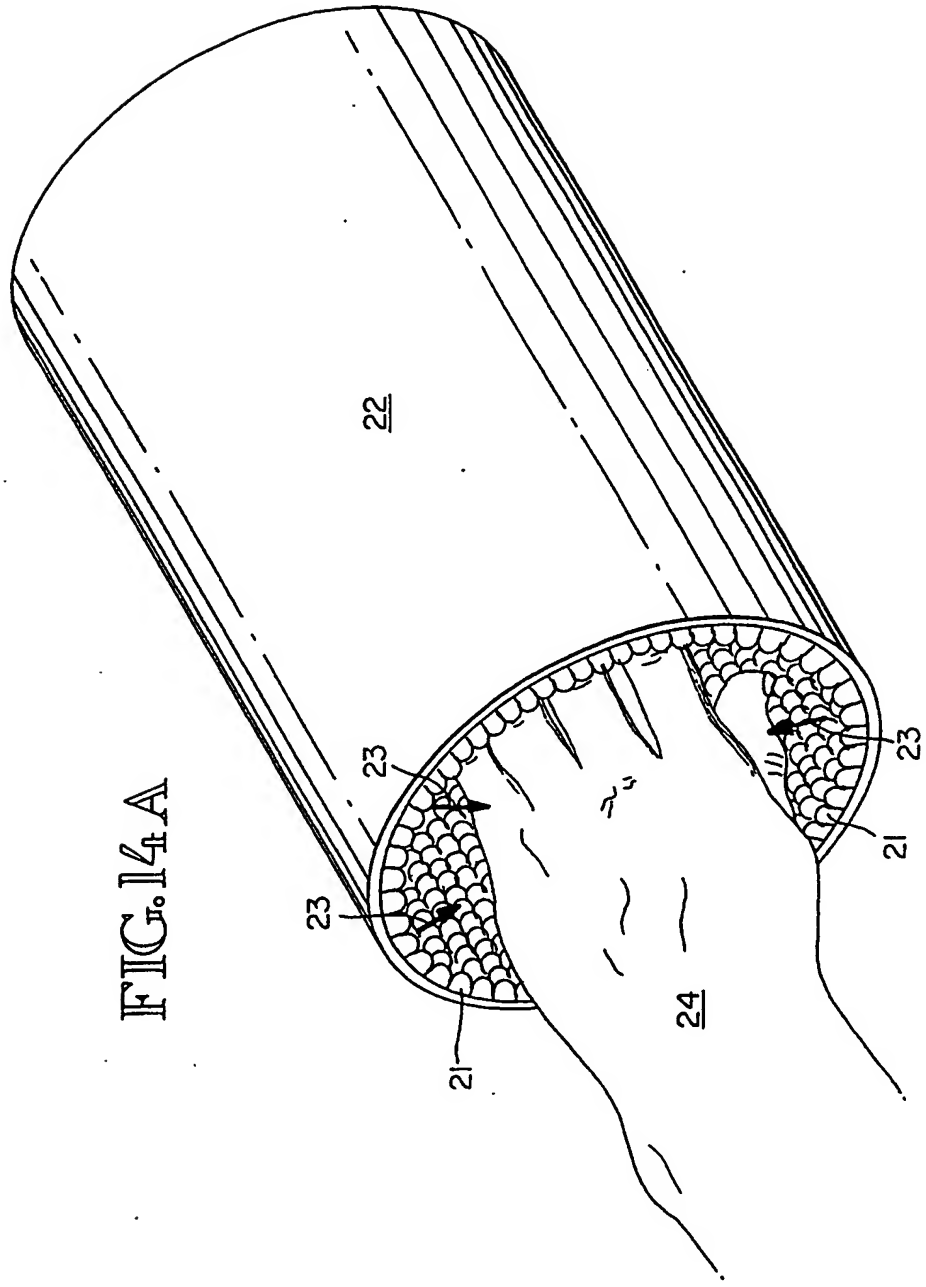


FIG. 14A

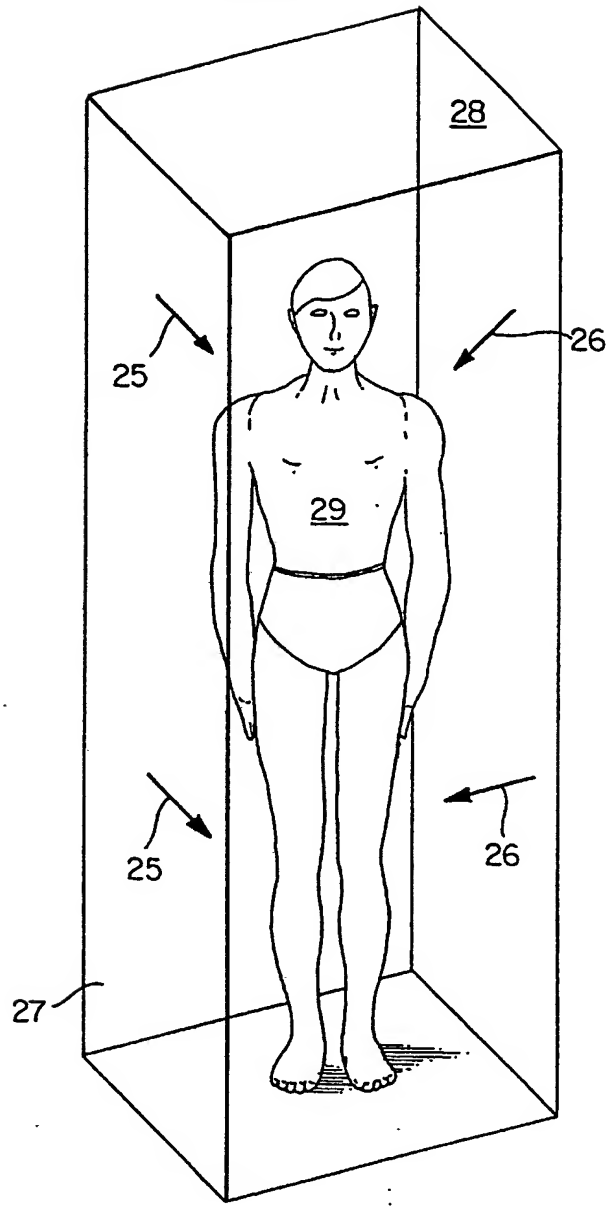
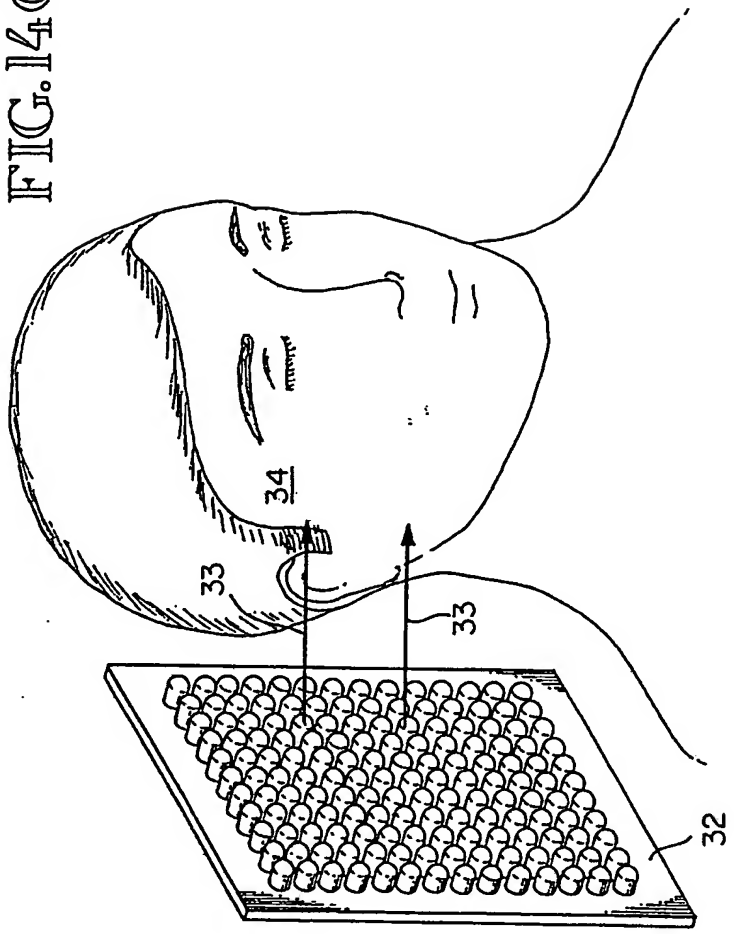


FIG. 14B

FIG. 14C



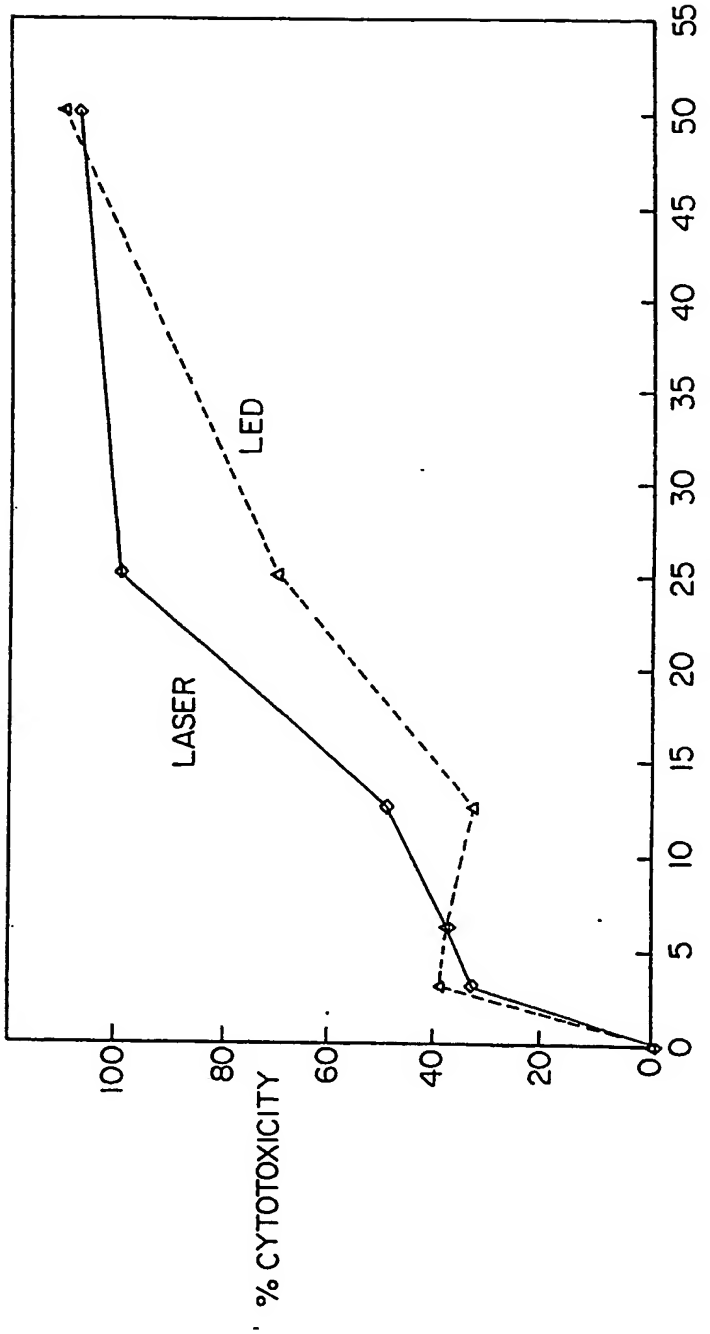
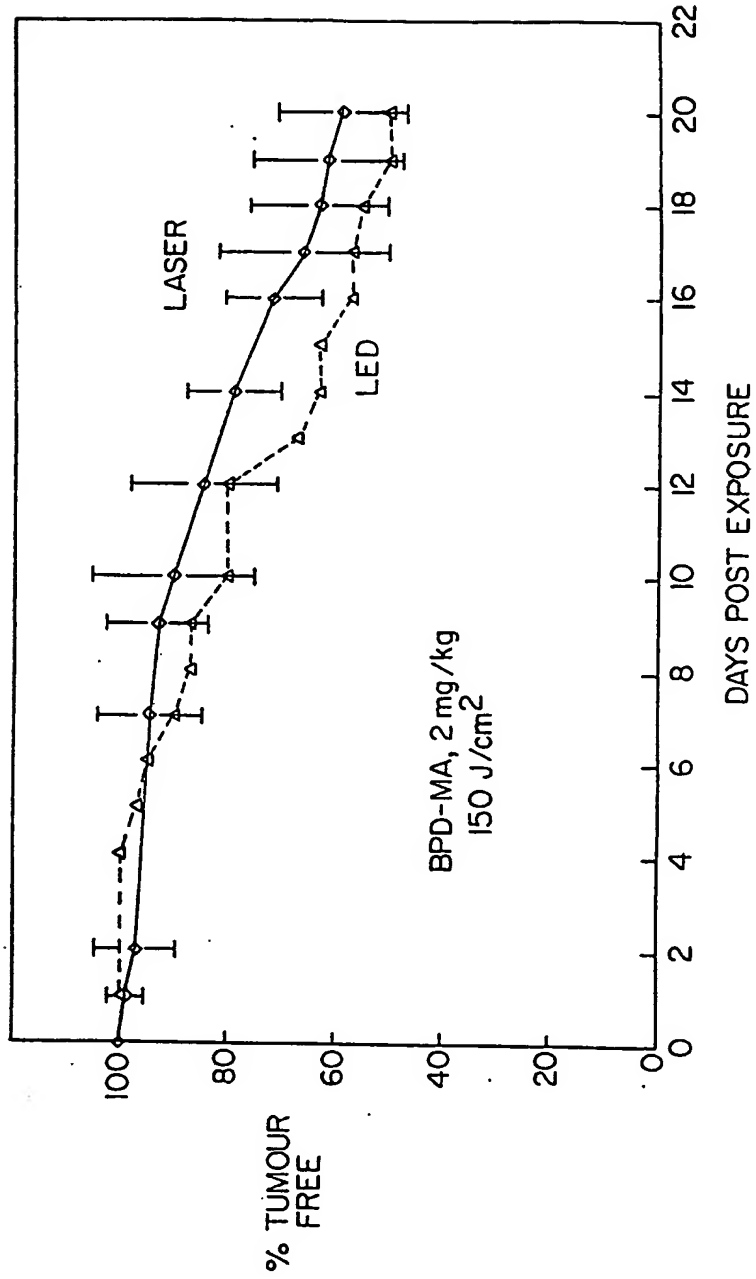

% CYTOTOXICITY
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
55
DRUG CONCENTRATION (ng/ml)
LASER
LED

FIG. 15

FIG. 16


LASER
LED
BPD-MA, 2 mg/kg
150 J/cm2
% TUMOUR FREE
DAYS POST EXPOSURE
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01893

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61B 17/36
US CL :128/396

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/396; 128/395, 396, 397; 604/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US, A, 4,822,335 (KAWAI ET AL.) 18 April 1989. See the entire document.	1, 9, 10, 14, 16, 17, 19, 21 2-8, 11-13, 15, 18 & 20
Y	GB, A, 2,212,010 (LISON ET AL.) 12 July 1989. See the entire document.	2-8, 11-13 15, 18 & 20
A,P	US, A, 5,132,101 (VOGEL ET AL.) 21 July 1992.	1-21
A,P	US, A, 5,179,120 (VOGEL ET AL.) 12 January 1993.	1-21
A	US, A, 4,305,390 (SWARTZ) 15 December 1981.	1-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search 26 MAY 1993	Date of mailing of the international search report 13 JUL 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer Debbie Wilson J. HARRISON Telephone No. (703) 308-2217

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01893

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,622,952 (GORDON) 18 November 1986.	1-21
A,P	US, A, 5,142,863 (DOUGHERTY ET AL.) 08 September 1992.	1-21
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A,P	US, A, 5,163,898 (MORCOS ET AL.) 17 November 1992.	1-21
A	EP, A, 02650338 (KUREMA KAGAKU KOGYO) 04 May 1988.	1-21
A	High Technology, 30 November 1984, p. 76, Herb Brody, "Laser Light Kills Marked Tumors" page 76.	1-21
A	Cancer, Research, vol. 38, August 1978, Dougherty et al., "Photoradiation Therapy for the Treatment of Malignant Tumors", pages 2628-2635.	1-21